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HISTOCHEMICAL CHANGES IN THE OVARIES OF
RESERPINE TREATED RATS

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MODIFICATIONS OF ESTERASES AND OF ALKALINE
PHOSPHATASES IN THE GENITAL TRACT
OF THE RAT AFTER TREATMENT WITH
RESERPINE

by

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A thesis presented to the Faculty
of the Yale University School of Medicine
in candidacy for the degree of
Doctor of Medicine

Department of Pathology, Yale University
School of Medicine



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MODIFICATIONS OF ESTERASES AND OF ALKALINE
PHOSPHATASES IN THE GENITAL TRACT
OF THE RAT AFTER TREATMENT WITH
RESERPINE

I. INTRODUCTION

It has been shown (1, 2, 3) that reserpine, alcohol extract of *Rauwolfia serpentina*, has a marked influence on the hypophyseal-genital system of the rat. Its administration rapidly suppresses vaginal keratinization and causes a slow involution of the ovary and of the uterus. The cytological aspect of the anterior hypophysis undergoes changes which suggest a disequilibrium of gonadotropic activity. One of the peculiarities of the genital reactions with treatment with reserpine occurs as the early disruption of the histological vaginal cycle and the relatively late histological changes in the ovaries. Histological examination does not reveal structural changes of the ovary until after eight to ten days of treatment.

It seemed interesting to carry out an histochemical study on the genital tract of the rat treated with reserpine. Verne (4, 5) and Verne and Herbert (6, 7) have shown that esterases and phosphatases furnish extremely precise information concerning the

functional activity of the corpus luteum. This work has been confirmed by Hunter and Kneiske (8). Therefore, a study of esterases and alkaline phosphatase, as well as mucopolysaccharide content and RNA and DNA concentration of cells in the ovary, vagina, and fallopian tubes, was undertaken in an attempt to discover and define changes in these tissues before they become evident by histological means.

II. TECHNIQUE

Female rats of the Wistar strain each weighing approximately 180 grams were treated daily with 50 gamma of Serpasil. Six groups of three animals were treated. The rats were sacrificed after three, five, seven, nine, twelve, and sixteen days of treatment. For controls, two groups of females were used. One group was offered a normal regimen. The second group was subjected to dietary restrictions. The study of the second group of controls seemed necessary in order to eliminate the possible effects of the 20 - 30% weight loss observed in the tested groups of rats. The rats in the underfed control group were sacrificed when they had lost forty per cent of their weight.

The studied organs were fixed in cold acetone.

The esterase studies were carried out with the tween 60 technique of Gomori. For the alkaline phosphatases the method of Gomori and Takamatsu was employed. The Unna and MacManus methods were used respectively for the nucleic acid and mucopolysaccharide studies.

III. RESULTS

A. Esterases (see pages 11, 12)

The corpora lutea in the control ovaries show a strongly positive reaction with the esterase stain. It is interesting to note that the intensity of the esterase reaction permits the age of the corpus luteum to be discerned. The best vascularized corpora with the least connective tissue, that is, the youngest ones, show the strongest reaction. The color varies from yellow-brown to yellow-gold. Under high power one sees that the colored droplets are dense at the periphery of the cell and and rarer around the nucleus.

For the female controls subjected to starvation, no alteration of the esterase activity can be noted. The ovaries present the same intensity of color. Starvation, therefore, does not play a role in the enzymatic activity of the ovary.

After three days of treatment with Serpasil, the

ovary already presents some clear modifications of esterase activity. In general, the intensity of the reaction has diminished about 50%. At the same time, the morphologic structure of the "yellow bodies" appear analogous to those of the controls. In certain ovaries one can observe a more marked attenuation of the esterase activity to the point that the old corpora are barely straw-colored. At high magnification one notices a significant diminution of peripheral granulations in the cell. This explains the weak intensity of the reaction. The diminution of the esterase activity begins in the central portion of the corpus luteum.

After five days of treatment, the esterase reaction appears more attenuated. The color of most of the corpora lutea is uniformly straw-colored. The distinction between the old corpora and the young ones can now only be made under high magnification. However, the ovaries from the animals sacrificed after seven days of treatment show a more intense esterase activity than those of five days of treatment. There still exists, however, a sharp distinction between these ovaries and those of the controls. But often the "yellow bodies" instead of being uniformly straw-colored contain some deep-staining granulations. The

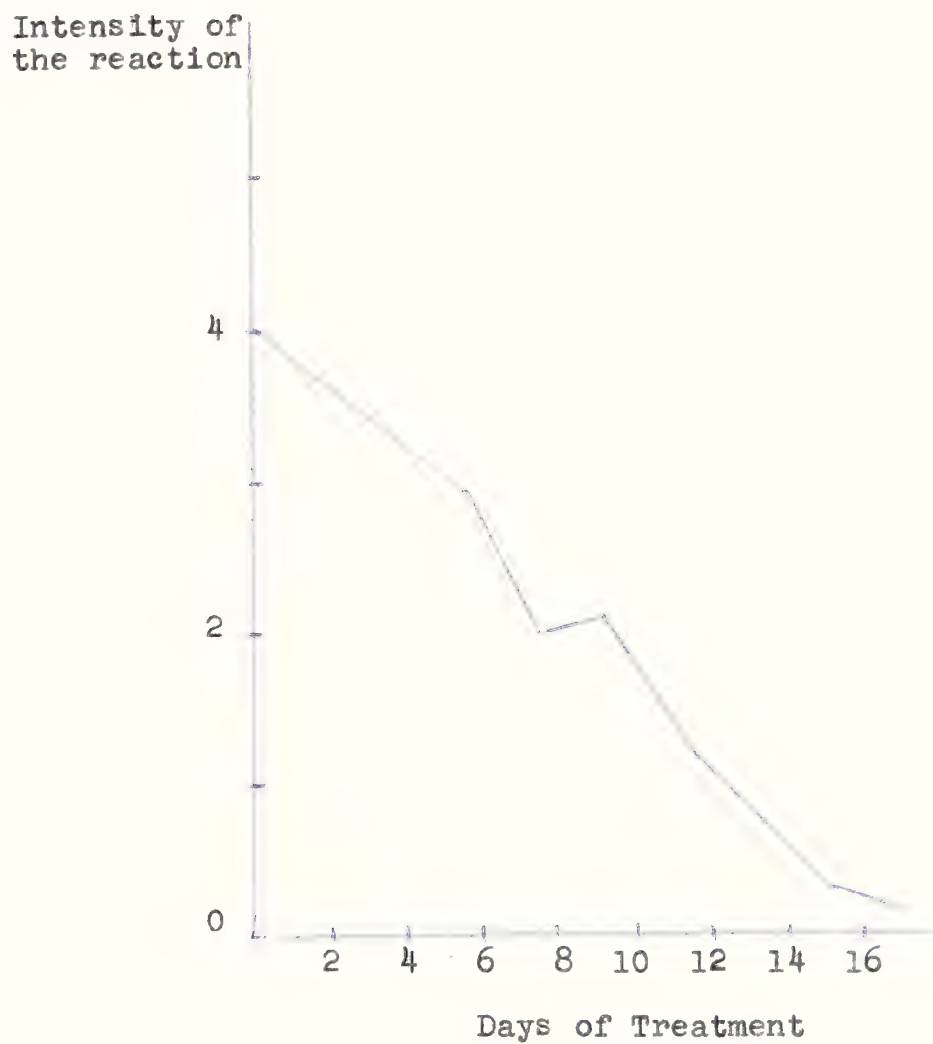
reasons for this accentuation of the reaction are not clear, but it is possible that it is a question of individual differences. It is interesting to note that Tuchmann-Duplessis et.al. (2) have shown individual variation with respect to the effect of Reserpine on the estrus cycle of the rat. In 5% of the cases, the cycle, instead of being completely suppressed after four days of treatment, reappears toward the tenth and even the fourteenth day.

The animals sacrificed the ninth day of treatment show once again a very weak esterase reaction in the majority of the corpora lutea. However, several of the "yellow bodies" display a mildly active reaction.

After twelve days of treatment the reaction is hardly visible with low power. At high magnification one can now see only several droplets in isolated corpora indicating esterase activity. The reaction is negative for all the others.

The reaction is almost entirely negative in the ovaries taken from the animals sacrificed after sixteen days of treatment. Occasional straw-colored droplets may be seen in the corpora that are young enough to have a rich vascular supply.

The variation of the esterase activity observed in the ovaries are schematically represented in the graph on page 6.



SCHEMATIC REPRESENTATION OF THE INTENSITY
OF THE ESTERASE ACTIVITY IN THE CORPORA
LUTEA OF RATS TREATED WITH RESERPINE

Intensity of
the reaction

2 4 6 8 10 12 14 16

Days of treatment

EXPERIMENTAL INVESTIGATION OF THE INFLUENCE
OF THE RESISTANCE ACTIVITY IN THE CORONA
OF A NEW TREATMENT WITH THE CORONA

The esterase activity in the uterus, fallopian tubes and vagina remained consistantly negative in the controls as well as the treated animals.

B. Phosphatases (see page 14)

The study of the alkaline phosphatases offers no information on the variations of ovarian function. Except for several slight and inconstant differences, the alkaline phosphatase reactions are the same in all tissues studied in the treated animals and in the controls.

The reaction is positive at the level of the theca interna, the granulosa cells, and in the walls of vessels, especially in the corpora lutea. The granulosa cells have a black nucleus and a gray cytoplasm. The lutein cells are clear.

The vaginal epithelium is intensely colored. The reaction manifests itself especially in the superficial layers of Malpighi.

In the treated animals, one sees an attenuation of the reaction which becomes noticeable under low power of magnification beginning on the seventh day. The deep layers of cells of the mucous membrane of the vagina remain colored whereas at the surface the cells

are only faintly colored gray.

In the uterus one observes a slight positive reaction along the walls of the vessels of the mucous membrane and occasionally in the nuclei of the uterine glands.

Thus the alkaline phosphatases of the genital tract does not seem to vary either quantitatively or with respect to their location.

C. Mucopolysaccharides and Nucleic Acids (see page 13)

In both the control animals and the treated ones the mucopolysaccharides and the nucleic acids exhibited no variation in intensity or location in any of the tissues examined.

IV. DISCUSSION

The study of the different enzymes of the ovary and of the genital tract shows only the esterase activity as being influenced by the administration of Reserpine. Very rapidly after three days of treatment and while the morphologic structure of the gonad appears still perfectly normal, the esterase reaction becomes strongly attenuated. This is therefore a clear indication of

ovarian hypofunction which removes the contradiction pointed out in the beginning of this work. The precocious perturbation of the cellular vaginal cycle which seemed to precede all morphologic alteration of the ovary appears in the light of the esterase reactions as the consequence of the gonadal hypoactivity. One finds here a new proof of the sensitivity of the esterase test for the appreciation of ovarian function.

It is also interesting to note that the characteristics of the other substances studied do not show alterations even when the ovary already presents morphologic modifications. The reasons for which the level of the alkaline phosphatases, nucleic acids and mucopolysaccharides do not vary remain obscure.

V. SUMMARY

Reserpine which disrupts the vaginal cycle of the rat induces simultaneously a marked diminution of esterase activity of the ovary. Three days after the institution of treatment, the esterase activity of the corpora lutea is approximately one-half as intense as the controls. The fall in intensity is slower between the fourth day and the seventh day and then once more

becomes accentuated until the twelfth day. At this time the esterase reaction is barely discernible or non-existent. The alkaline phosphatases, the nucleic acids and the mucopolysaccharides, however, do vary during the course of treatment.

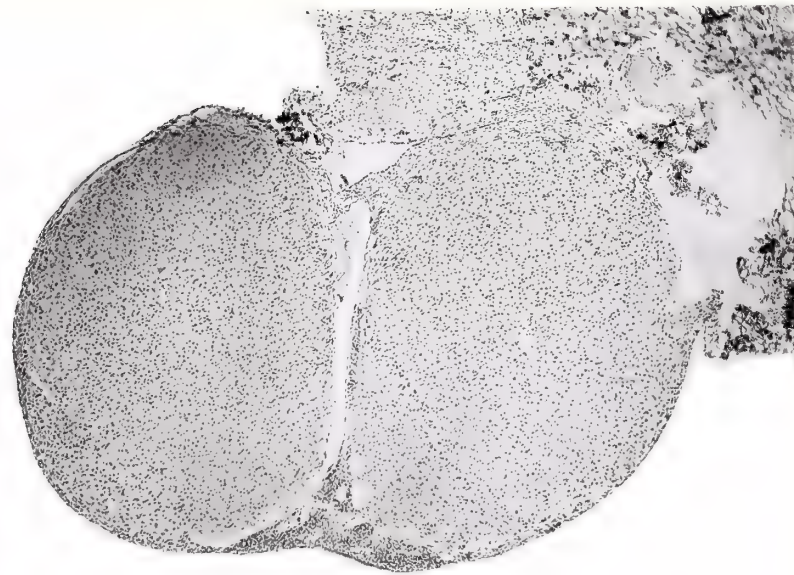
APPENDIX



Section of uterus stained for
esterases. Control animal.



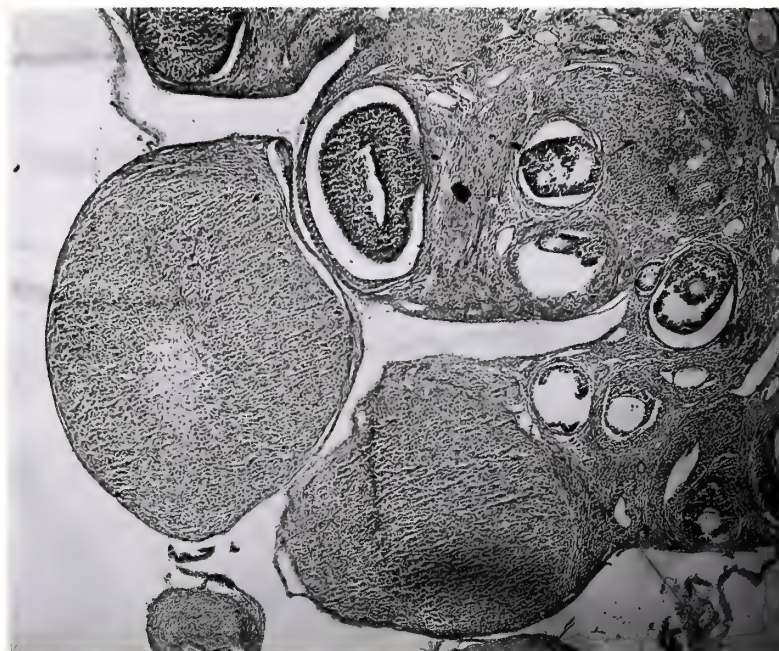
Section of uterus stained for
esterases. Starved animal
control.



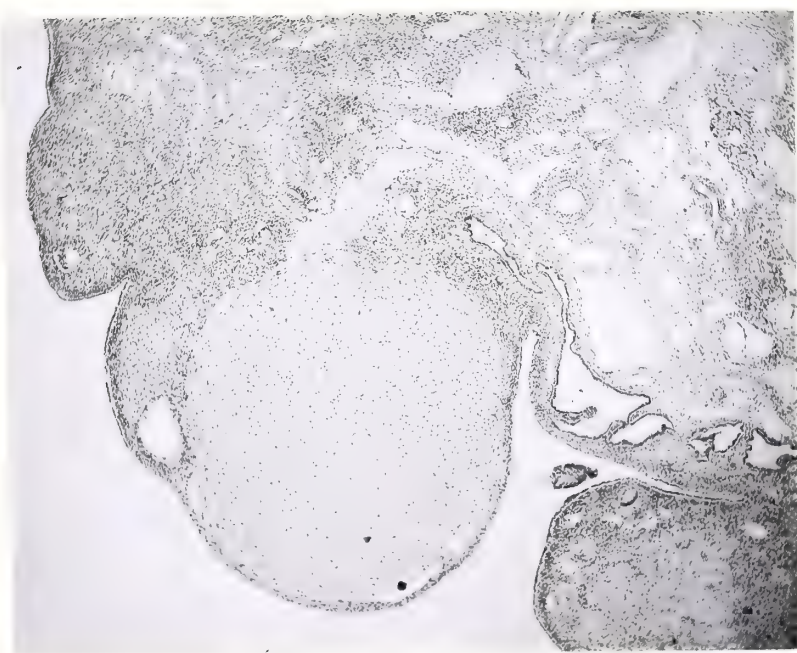
Section of uterus stained for esterases. Animal treated for three days with Reserpine.



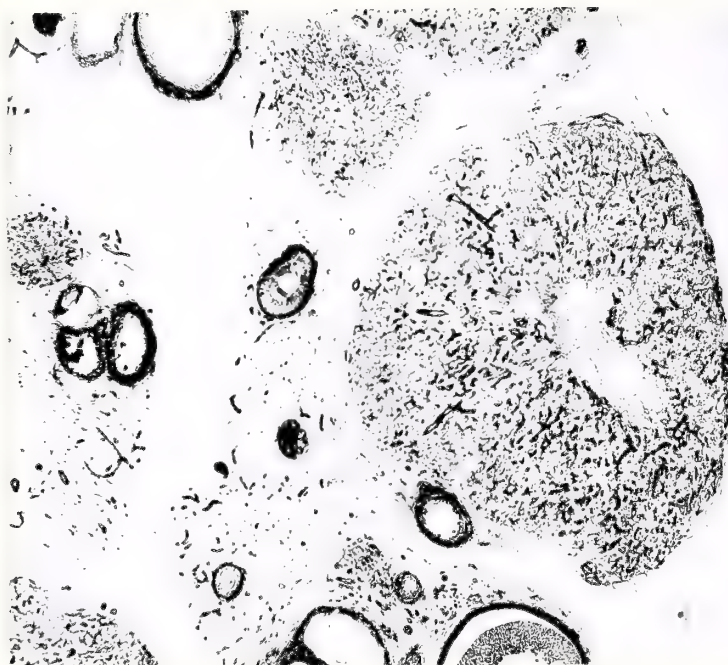
Section of uterus stained for esterases. Animal treated for sixteen days with Reserpine.



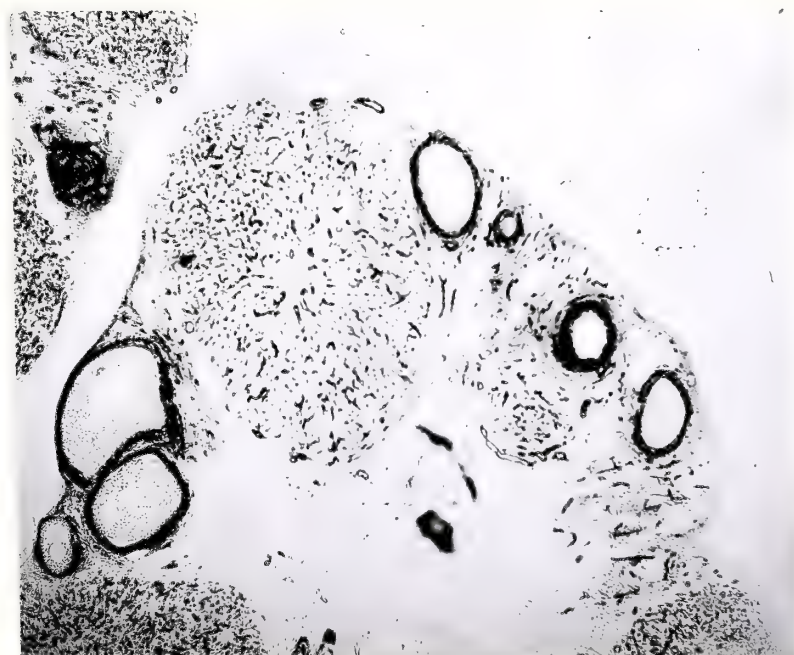
Section of uterus stained for
mucopolysaccharides. Animal
treated for sixteen days.



Section of uterus stained for
mucopolysaccharides. Control
animal.



Section of uterus stained for alkaline phosphatases. Animal treated for sixteen days.



Section of uterus stained for alkaline phosphatases. Control animal.

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